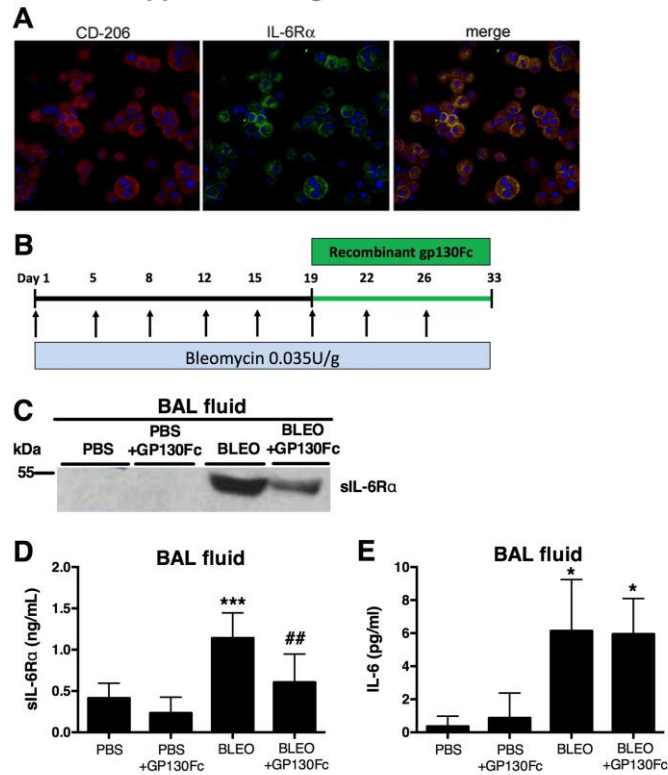


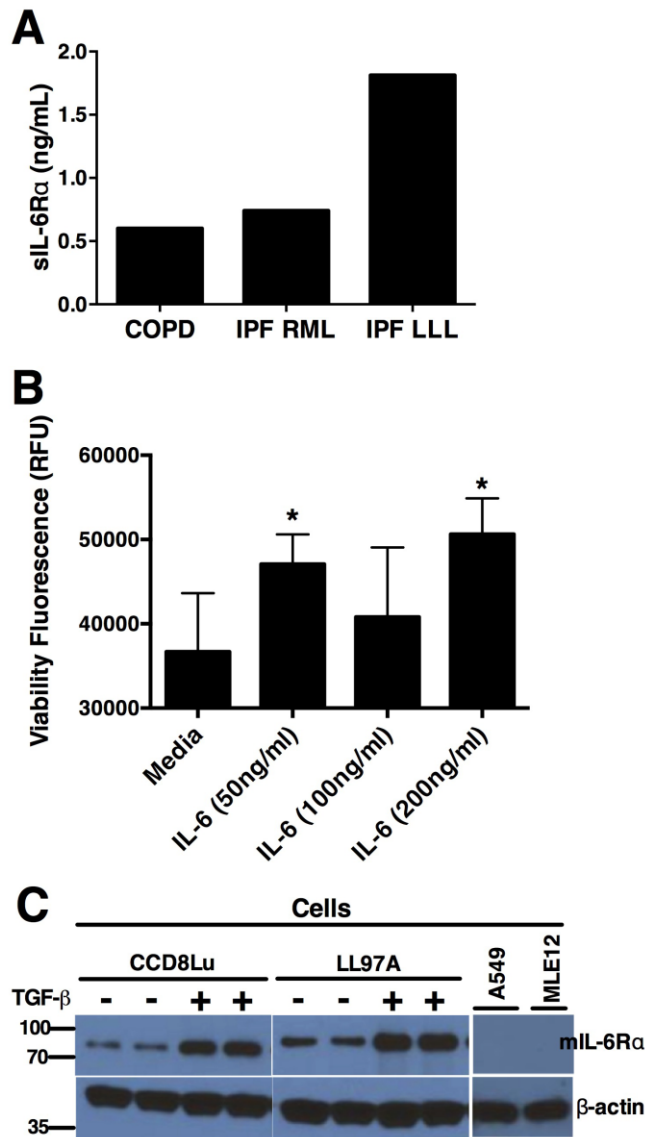
Supplemental Figures

Le et al, Supplemental Figure 1



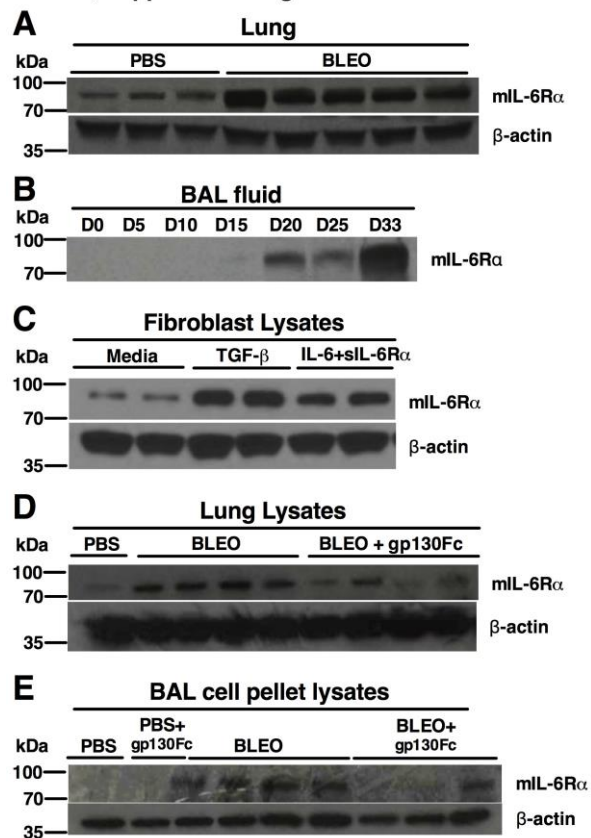
Supplemental Figure 1. (A) Dual immunofluorescence to detect mIL-6Rα on macrophages isolated from IPF patients; CD-206 (red) = marker of M₂ macrophages, human IL-6Rα (green), dapi (blue). (B) Experimental protocol for *in vivo* neutralization of sIL-6Rα. Wildtype C57Blk6 male mice were injected intraperitoneally with saline or bleomycin twice weekly for 4 weeks. Beginning on day 19, when pulmonary fibrosis has been established, daily treatment with vehicle (saline) or recombinant gp130Fc was performed. Mice were sacrificed and samples collected on day 33. (C) Western blot analysis and (D) ELISA measurement of sIL-6Rα levels in BAL fluid from wild type C57Blk6 mice given saline or bleomycin with and without gp130Fc, day 33. (E) ELISA quantification of IL-6 levels in BAL fluid. All data presented as mean ± SEM, n≥6 for B & D, n≥3 for E. *significant difference from PBS-treated cohort; #significant difference from bleomycin-exposed mice. * = p<0.05, ** = 0.001<p<0.01, *** = p<0.001.

Le et al, Supplemental Figure 2



Supplemental Figure 2. (A) Soluble IL-6Rα expression was assessed in patients with Idiopathic Pulmonary Fibrosis. ELISA quantification of sIL-6Rα in BAL fluid from non-fibrotic (RML) and fibrotic (LLL) lobes of an IPF patient was performed. (B) Proliferation of control fibroblasts to increasing concentrations of IL-6. (C) Western blot analysis of mIL-6Rα in protein lysates from control and IPF fibroblast cell lines (CCD8Lu and LL97A, +/- TGF-β) and type II pneumocyte cell lines (A549 & MLE-12).

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Supplemental Figure 3. Membrane IL-6R α expression was assessed in mice with pulmonary fibrosis. Western blot analysis of mIL-6R α in (A) Day 33 lung lysates and (B) BAL fluid samples over the course of the IPB model. (C) Effect of IL-6 *trans* signaling on expression of mIL-6R α in control fibroblasts. Normal fibroblasts were serum-starved for 24 hours and then stimulated with IL-6 alone or IL-6 and siIL-6R α for 48 hours. Changes in membrane IL-6R alpha following chronic bleomycin exposure in mice treated with recombinant gp130Fc were also examined. Wildtype C57Blk6 male mice were injected intraperitoneally with saline or bleomycin twice weekly for 4 weeks. Beginning on day 19, when pulmonary fibrosis has been established, daily treatment with vehicle (saline) or recombinant gp130Fc was performed. Mice were sacrificed and samples collected on day 33. Western blot analysis of mIL-6R α expression in (D) whole lung and (E) BAL cell pellet protein lysates.